spans the viral membrane and contains at its amino-terminus a sequence of amino acids important for the fusion of viral and cellular membranes. The HIV envelope glycoproteins assemble as noncovalent oligomers, almost certainly trimers, of gp120/gp41 on the virus surface. The detailed events of viral entry remain poorly understood but involve gp120 binding first CD4 then a fusogenic chemokine receptor, followed by gp41-mediated virus-cell fusion.

Because of their location on the virion surface and central role in mediating viral entry, the HIV envelope glycoproteins provide important targets for HIV vaccine development. Although most HIV-infected individuals mount a robust antibody (Ab) response to the envelope glycoproteins, most anti-gp120 and anti-gp41 Abs produced during natural infection bind weakly or not at all to virions and are thus functionally ineffective. These Abs are probably elicited and affinity matured against "viral debris" comprising gp120 monomers or improperly processed oligomers released from virions or infected cells. (Burton and Montefiori, AIDS, 11 [Suppl A]: 587, 1997)

Several preventive HIV-1 subunit vaccines have been tested in Phase I and II clinical trials and a multivalent formulation is entering Phase III testing. These vaccines have contained either monomeric gp120 or unprocessed gp160 proteins. In addition, the vaccines mostly have been derived from viruses adapted to grow to high levels in immortalized T cell lines (TCLA viruses). These vaccines have consistently elicited Abs which neutralize the homologous strain of virus and some additional TCLA viruses. However, the Abs do not potently neutralize primary HIV-1 isolates (Mascola *et al.*, J. Infec. Dis. 173:340, 1996). Compared with TCLA strains, the more clinically relevant primary isolates typically possess a different cellular tropism, show a

protein encoded by the above recombinant nucleic acid molecule. The antibody may be of the IgM, IgA, IgE or IgG class or subclasses thereof. The above antibody fragment includes but is not limited to Fab, Fab', (Fab')2, Fv and single chain antibodies.

This invention provides an isolated antibody light chain of the above antibody, or fragment or oligomer thereof. This invention also provides an isolated antibody heavy chain of the above antibody, or fragment or oligomer thereof. This invention also provides one or more CDR regions of the above antibody. In one embodiment, the antibody is derivatized. In another embodiment, the antibody is a human antibody. The antibody includes but is not limited to monoclonal antibodies and polyclonal antibodies. In one embodiment, antibody is humanized.

This invention provides an isolated nucleic acid molecule encoding the above antibody.

This invention provides a method of reducing the likelihood of a virally exposed subject from becoming infected with the virus comprising administering the above antibody or the above isolated nucleic acid, thereby reducing the likelihood of the subject from becoming infected with the virus.

This invention provides a method of treating a subject infected with a virus comprising administering the above antibody or the above isolated nucleic acid, thereby treating the subject. In a preferred embodiment, the virus is HIV.

This invention provides an agent capable of binding the mutant viral envelope protein encoded by the above recombinant nucleic acid molecule. In one embodiment, the agent inhibits viral infection.

Figure 11

Formation of an intersubunit cysteine bridge in envelope proteins with deletions in variable loop regions. a) The ΔV1V2*V3 protein and the ΔV1V2*V3 N357C N398Q protein with two cysteines at positions 492 and 596 (indicated with CC) were precipitated with 2G12 and F91 (lanes 3 & 7 and 4 & 8, respectively). The appropriate controls without cysteine mutations are shown in lanes 1, 2, 5 & 6. The wild-type protein without extra cysteines is shown in lanes 9 and 10. All the proteins were cleaved by furin, except for the wild-type protein of lane 10. The approximate sizes in kDa are given on the right. b) Various loop deleted proteins with two cysteines at positions 492 and 596 (CC) were precipitated with 2G12 (lanes 3, 5, 7, 9, 11 & 13). Proteins with the same deletions without extra cysteines are given in the adjacent lanes. These control proteins were not cleaved by furin. The full-length SOS gp140 protein is included as a control in lane 1.

Figure 12

Antigenic characterization of the A492C/T596C mutant in combination with deletions in the variable loops. All mutants were expressed in the presence of exogenous furin. The Abs used in RIPAs are indicated on top. a) The A492C/T596C ΔV1V2* mutant and b) the A492C/T596C ΔV3 mutant.

Figure 13

Nucleotide (A) and amino acid (B) sequences for HIV-1_{JR-FL} SOS gp140. The amino acid numbering system corresponds to that for wild-type JR-FL (Genbank Accession #U63632). The cysteine mutations are indicated in underlined bold type face.

Figure 14

to cysteine. Because of the sequence variability of HIV, this amino acid will not be at position 492 in all other HIV isolates. For example, in HIV-1_{NL4-3} the corresponding amino acid is A499 (Genbank Accesion # AAA44992). It may also be a homologous amino acid other than alanine or cysteine. This invention encompasses cysteine mutations in such amino acids, which can be readily identified in other HIV isolates by those skilled in the art.

As used herein, "T596C mutation" refers to a point mutation of amino acid 596 in HIV-1_{JR-FL} gp41 from threonine to cysteine.

Because of the sequence variability of HIV, this amino acid will not be at position 596 in all other HIV isolates. For example, in HIV-1_{NL4-3} the corresponding amino acid is T603 (Genbank Accesion # AAA44992). It may also be a homologous amino acid other than threonine or cysteine. This invention encompasses cysteine mutations in such amino acids, which can be readily identified in other HIV isolates by those skilled in the art.

In another embodiment, a cysteine in the C1 region of gp120 is disulfide linked to a cysteine in the ectodomain of gp41.

As used herein, "C1 region" means the first conserved sequence of amino acids in the mature gp120 glycoprotein. The C1 region includes the amino-terminal amino acids. In HIV_{JR-FL}, the C1 region consists of the amino acids VEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPN PQEVVLENVTEHFNMWKNNMVEQMQEDIISLWDQSLKPCVKLTPLCVTLN. Amino acid resides 30-130 of the sequence set forth in in figure 3A have this sequence. In other HIV isolates, the C1 region will comprise a homologous amino-terminal sequence of amino acids of similar length. W44C and P600C mutations are as defined above for A492 and T596 mutations. Because

includes but is not limited to gp41. An amino acid in the ectodomain of gp41 may be mutated. The amino acids residues which may be mutated include but are not limited to the following amino acid residues: D583; W587; T595; V599; and P600. The gp41 amino acid residues are also set forth in Figure 3B.

This invention provides a mutant viral envelope protein which differs from the corresponding wild type protein in at least one amino acid which yields a complex comprising a surface protein and a transmembrane protein which has enhanced stability relative to the corresponding complex obtained from the wild type envelope protein, wherein the surface protein and transmembrane protein are encoded by different nucleic acids.

This invention provides a complex comprising a viral surface protein and a viral transmembrane protein which has enhanced stability relative to the corresponding complex obtained from the wildtype envelope protein, yielded by the proteolysis of a mutant viral envelope protein with a sequence which differs from the corresponding wild type protein sequence in at least one amino acid, wherein the surface protein and transmembrane protein are encoded by different nucleic acids.

This invention provides a nucleic acid which encodes a mutant surface protein wherein the surface protein is complexed with its corresponding transmembrane protein and will have enhanced stability.

This invention provides a nucleic acid which encodes a mutant transmembrane protein wherein the transmembrane protein is complexed with its corresponding surface protein and will have enhanced stability.

This invention provides an antibody which binds to the

Sodroski, J. Virol. 70:1863, 1996); Mab 212A to a conformational C1-C5 epitope (Moore et al. J. Virol 68:6836, 1994); Mab 17b to a CD4-inducible epitope (Moore and Sodroski, J. Virol. 70:1863, 1996); Mab A32 to a CD4-inducible C1-C4 epitope (Moore and Sodroski, J. Virol. 70:1863, 1996; Sullivan et al, J. Virol. 72:4694, 1998); Mabs G3-519 and G3-299 to C4 or C4/V3 epitopes (Moore and Sodroski, J. Virol. 70:1863, 1996). Mabs to gp41 epitopes included 7B2 to epitope cluster 1(kindly provided by Jim Robinson, Tulane University); 25C2 to the fusion peptide region (Buchacher et al. AIDS Res. Human Retrov. 10:359, 1994); 2F5 to a neutralizing epitope encompassing residues 665-690 (Munster et al. J. Virol. 68:4031, 1994). The tetrameric CD4-IgG2 has been described previously (Allaway et al. AIDS Res. Human Retrovir. 11:533, 1995).

Anti-HIV Abs were obtained from commercial sources, from the NIH AIDS Reagent Program, or from the inventor. Where indicated, the Abs were biotinylated with NHS-biotin (Pierce, Rockford, IL) according to the manufacturer's instructions.

Monomeric $gp120_{JR-FL}$ was produced in CHO cells stably transfected with the PPI4-tPA-$gp120_{JR-FL}$ plasmid as described (U.S. Patents 5,866,163 and 5,869,624). Soluble CD4 was purchased from Bartels Corporation (Issaquah, WA).

2. Construction of PPI4-based plasmids expressing wild-type and mutant HIV envelope proteins

Wild-type gp140s (gp140WT) The gp140 coding sequences were amplified using the polymerase chain reaction (PCR) from full-length molecular clones of the HIV-1 isolates JR-FL, DH123, Gun-1, 89.6, NL4-3 and HxB2. The 5' primer used was designated Kpn1env (5'-GTCTATTATGGGGTACCTGTGTGGAA AGAAGC-3') while the 3' primer was BstB1env (5'-

CGCAGACGCAGATTCGAATTAATACCACAGCCAGTT-3'). PCR was performed under stringent conditions to limit the extent of *Taq* polymerase-introduced error. The PCR products were digested with the restriction enzymes *Kpn*1 and *Xho*1 and purified by agarose gel electrophoresis. Plasmid PPI4-tPA-$gp120_{JR-FL}$ was also digested with the two restriction enzymes and the large fragment (vector) was similarly gel-purified. The PPI4-tPA-$gp120_{JR-FL}$ expression vector has been described previously (Hasel and Maddon, U.S. Patents #5886163 and 5869624). Ligations of insert and vector were carried out overnight at room temperature. DH5αF'IQ10 bacteria were transformed with 1/20 of each ligation. Colonies were screened directly by PCR to determine if they were transformed with vector containing the insert. DNA from three positive clones of each construct were purified using a plasmid preparation kit (Qiagen, Valencia, CA) and both strands of the entire gp160 were sequenced. By way of example, pPPI4-$gp140WT_{JR-FL}$ and pPPI4-$gp140WT_{DH123}$ refer to vectors expressing wild-type, cleavable gp140s derived from $HIV\text{-}1_{JR-FL}$ and $HIV\text{-}1_{DH123}$, respectively.

gp140UNC A gp120-gp41 cleavage site mutant of JR-FL gp140 was generated by substitutions within the REKR motif at the gp120 C-terminus, as described previously (Earl et al., Proc. Natl. Acad. Sci. USA 87:648, 1990). The deletions were made by site-directed mutagenesis using the mutagenic primers 5'140M (5'-CTACGACTTCGTCTCCGCCTT CGACTACGGGGAATAGGAGCTGTGTTCCTTGGGTTCTTG-3') and 3'gp140M (sequence conjunction with Kpn1env and BstB1env 5'-TCGAAGGCGGAGACGAAGTCGTAGCCGCAGTGCCTTGGTGG GTGCTACTCCTAATGGTTC-3'). In conjunction with Kpn1env and BstB1, the PCR product was digested with *Kpn*1 and *Bst*B1 and subcloned into pPPI4 as described above.

Loop-deleted gp120s and gp140s PPI4-based plasmids

expressing variable loop-deleted forms of gp120 and gp140 proteins were prepared using the splicing by overlap extension method as described previously (Binley *et al.*, AIDS Res. Human Retrovir. 14:191, 1998). In the singly loop-deleted mutants, a Gly-Ala-Gly spacer is used to replace D132-K152 (ΔV1), F156-I191 (ΔV2), or T300-G320 (ΔV3). The numbering system corresponds to that for the JR-FL clone of HIV-1 (Genbank Accession # U63632).

PCR amplification using DGKPN5'PPI4 and 5JV1V2-B (5'-GTCTATTATGGGGTACCTGTGTGGAAAGAAGC-3') on a ΔV1 template and subsequent digestion by *Kpn*1 and *Bam*H1 generated a 292bp fragment lacking the sequences encoding the V1 loop. This fragment was cloned into a plasmid lacking the sequences for the V2 loop using the Kpn1 and BamH1 restriction sites. The resulting plasmid was designated ΔV1V2' and contained a Gly-Ala-Gly sequences in place of both D132-K152 and F156-I191. Envs lacking the V1, V2 and V3 loops were generated in a similar way using a fragment generated by PCR on a ΔV3 template with primers 3JV2-B (5'-GTCTGAGTCGGATCCTGTGACACCTCAGTCATTACACAG-3')and H6NEW (5'CTCGAGTCTTCGAATTAGTGATGGGTGATGGTGATGATACCACAGCCATTTT GTTATGTC-3'). The fragment was cloned into ΔV1V2', using BamH1 and BstB1. The resulting env construct was named ΔV1V2'V3. The glycoproteins encoded by the ΔV1V2' and ΔV1V2'V3 plasmids encode a short sequence of amino acids spanning C125 to C130. These sequences were removed using mutagenic primers that replace T127-I191 with a Gly-Ala-Gly sequence. We performed PCR amplification with primers 3'DV1V2STU1 (5'-GGCTCAAAGGATATCTTTGGACAGGCCTGT GTAATGACTGAGGTGTCACATCCTGCACCACAGAGTGGGGTTAATTTTACACATGGC-3') and DGKPN5'PPI4, digested the resulting fragment by *Stu*1 and *Kpn*1 and cloned it in a PPI4 gp140 vector. The resulting gp140 was named ΔV1V2*. In an analogous manner ΔV1V2*V3 was constructed. The amino acid substitutions are shown schematically in Figure 10.

To minimize the production of gp140NON, pcDNA3.1-furin and pPPI4-gp140WT$_{JR-FL}$ were cotransfected into 293T cells, and RIPA assay was performed using the anti-gp120 MAb 2G12. As indicated in Figure 2, furin eliminated production of gp140NON but had no effect on gp140UNC. Similar results were obtained in RIPAs performed using other anti-gp120 MAbs (data not shown).

Treatment of the samples with DTT prior to SDS-PAGE did not affect the migration or relative amounts of these bands, indicating that the gp140s consist of a single polypeptide chain rather than separate gp120-gp41 molecules linked by an adventitious disulfide bond.

2. Stabilization of the gp120-gp41 interaction by introduction of double cysteine mutations

With furin co-transfection, we could now express a soluble gp140 protein in which the gp120 and gp41ECTO components were associated only through a non-covalent linkage, mimicking what occurs in the native trimeric envelope glycoprotein complex on virions. However, on virions or the surface of infected cells, the gp120-gp41 association is weak, so that gp120 is gradually shed (McKeating et al. J. Virol 65:852, 1991). We found this to occur also with the gp140WT protein made in the presence of endogenous furin. Thus, we could detect very little, if any, stable gp120-gp41ECTO complexes in the supernatants from gp140WT-expressing cells after immunoprecipitation. We therefore sought ways to stabilize the non-covalent gp120-gp41 interaction, by the introduction of an intermolecular disulfide bond between the gp120 and gp41 subunits.

We therefore substituted a cysteine residue at one of

generated double-cysteine mutants of gp140's from other HIV-1 strains. These include the R5X4 virus DH123 and the X4 virus HxB2. In each case, the cysteines were introduced at the residues equivalent to alanine 492 and threonine 596 of JR-FL. The resulting SOS proteins were transiently expressed in 293T cells and analyzed by RIPA to ascertain their assembly, processing and antigenicity. As indicated in Fig. 9, 140 kDa material is formed efficiently in the DH123 and HxB2 SOS proteins, demonstrating that our methods can successfully stabilize the envelope proteins of diverse viral isolates.

6. Disulfide stabilization of HIV envelope proteins modified in variable loop and glycosylation site regions

Since there is evidence to suggest that certain variable loop and glycosylation site mutations provide a means to better expose underlying conserved neutralization epitopes, we examined the assembly and antigenicity of disulfide-stabilized forms. In initial studies, A492C/T596C JR-FL gp140 mutants were created for each of the ΔV1, ΔV2, ΔV3, ΔV1V1*, and ΔV1V2*V3 molecules described above. For the ΔV1V2*V3 protein, glycosylation site mutants were also synthesized by N-Q point mutations of amino acids 357 and 398.

For each of the singly and doubly loop-deleted mutants, we could detect gp140 bands in comparable quantities as for the full-length SOS gp140 protein (Fig. 11B). To see whether deletion of the variable loops altered antigenicity in an oligomeric context, we precipitated the ΔV3 and ΔV1V2* SOS proteins with a panel of MAbs (Fig. 12). MAbs to gp41 except 2F5 did not bind to loop deleted versions of the cysteine stabilized protein, indicating